Rostrocaudal polarity formation of chick optic tectum

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ABSTRACT The optic tectum receives retinal fibers in a topographically ordered manner. For the formation of the precise connections, the tectum is believed to be positionally specified by gradients of molecules along axes. Rostrocaudal polarity of the tectum is first detectable at embryonic day 2 (E2) in the chick, by the caudorostral gradient of *en* expression, then by the rostrocaudal gradient of cytoarchitectonic development. Tectum rotation experiments showed that tectum rostrocaudal polarity is not determined at around 10-somite stage, but is fixed on E3. Ectopic tectum was produced in the diencephalon by transplanting the mesencephalic alar plate heterotopically. In the ectopic tectum, *en* expression was weakest at the caudal (nearest to the host diencephalo-mesencephalon junction) and strongest at the rostral end. Consequently, the pattern of *en* expression in the host and ectopic tecta was nearly a mirror image. Retinal fibers projected to the ectopic tectum in a topographic order in accordance with the inverted gradient of the *en* expression pattern. Ectopic tecta was also produced by heterochronal transplantations between E3 host and E2 donor, where the *en* pattern was preserved. Retinotectal projection pattern was also preserved, suggesting that *en* expression patterns are followed by retinotopic order with regard to rostrocaudal polarity.

KEY WORDS: *optic tectum, rostrocaudal polarity, engrailed retinotectal projection*

Introduction

Optic tectum is a visual center of lower vertebrates, and receives retinal fibers in an exact retinotopic manner. Fibers from nasal part of the retina project to the caudal tectum, and those from temporal part of the retina project to the rostral part of the tectum (Crossland and Uchwat, 1979). For the precise neural circuit formation, Sperry (1963) proposed a chemoaffinity theory. For the retinotectal circuit formation, it is now accepted that the positional specification of both tectal and retinal cells are represented by gradients along axes rather than by different labeling of each point. Thus retina and tectum should be positionally specified by the time retinal fibers reach the tectum. In this review, we will focus on the development of the rostrocaudal polarity of the avian optic tectum.

Developmental events concerning rostrocaudal polarity formation of the tectum

The main role of the optic tectum is to receive retinal fibers in a retinotopic manner. Hence the final positional specification of the tectum may be represented by one that retinal fibers read to find their target. There are some events concerning rostrocaudal polarity, and the final positional specification may be established through a cascade of these developmental events.

The first developmental event along the rostrocaudal axis of the tectum known so far is expression of *engrailed (en). en* is a homolog of *Drosophila* segment polarity gene *engrailed*, and contains homeobox. In chick, 2 *engrailed* genes are identified, *en-1* and *en-2* (Logan *et al.*, 1992). *en-2* is detected from E2 (embryonic day 2) by a monoclonal antibody, 4D9. We used 4D9 antibody to detect *en* expression in a series of our experiments. *en* is expressed strongly at the mesencephalo-metencephalon junction, and weakened toward the diencephalo-mesencephalon junction (Fig. 1). In other words, *en* is expressed in a caudorostral gradient (Gardner *et al.*, 1988; Patel *et al.*, 1989). From about E5, there is a rostrocaudal gradient of cytoarchitecture. Rostral part of the tectum differentiates faster and has more tectal laminas than the caudal (LaVail and Cowan, 1971). Finally, the tectal surface is positionally specified along a rostrocaudal axis by E8 (Walter *et al.*, 1987a,b), when the retinal fibers invade the tectum. The retinal fibers may read the difference to find their target.

Plasticity in rostrocaudal polarity of the tectum

It is an interesting question whether the polarity of the tectum is determined from an early stage of development. To answer this question, rotation of tectal anlagen was performed. A quail tectum anlage was transplanted into a chick mesencephalon by rotating its rostrocaudal axis 180° at about 10-somite stage.

Development of the rotated tectum proceeded similarly to the contralateral host tectum. Twenty-four hours after the rotation, the *en* expression pattern was already regulated to the host pattern (Martinez and Alvarado-Mallart, 1990). *en* was expressed strongly at the caudal part of the rotated tectum (though it was originally

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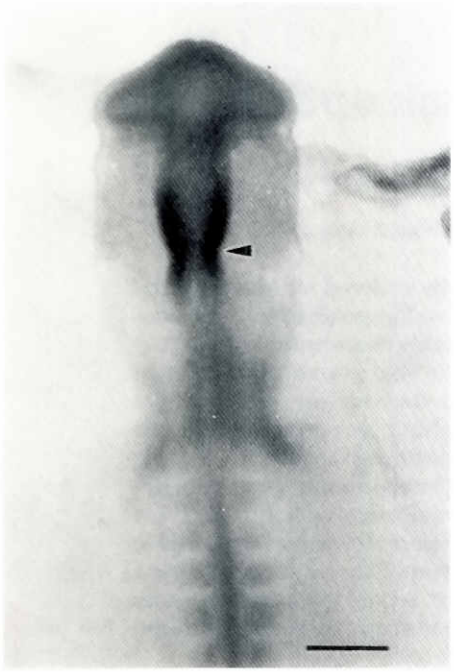

Fig. 1. E2 chick embryo stained *in toto* with anti*engrailed* protein, 4D9. *The staining is strong near the mes-metencephalon junction (arrowhead) and weakened toward rostral. Bar, 200 μm.*

rostral), and weakly at the rostral part. Rostral part of the rotated tectum (original caudal) differentiated faster than the caudal and received temporal retinal fibers (Ichijo *et al.*, 1990; Matsuno *et al.*, 1991).

The caudal part of the rotated tectum received the nasal retinal fibers, and the rostral part of the rotated tectum received temporal retinal fibers. These results indicate that the rostrocaudal polarity of the optic tectum is not fixed at around the 10-somite stage, and that it is established through interaction with surrounding tissues.

Then we checked when the polarity of the tectum anlagen is fixed. For this purpose, we performed tectum rotation experiment at E3. Since it is very difficult to rotate whole tectum, we took caudal half of quail left tectum and transplanted into the rostral part of the chick right tectum (double caudal tectum) (Fig. 2A). In a similar way, double rostral tectum was produced at E3.

When the operation was performed after the 20-somite stage, the transplant was not regulated by the host, but kept its original characteristics of *en* expression. Original caudal tectum anlagen kept expressing *en* strongly at the rostral part of the tectum (Itasaki *et al.*, 1991). On the other hand, original rostral tectum anlagen did not express *en* strongly at the caudal part of the tectum. On the double caudal tectum in which *en* had been expressed strongly at the rostral part, nasal retinal fibers were attracted by the transplant (Itasaki *et al.*, 1991). On the double rostral tectum, in which *en* had not been expressed strongly at the caudal part, the nasal retinal fibers extended to the caudal part, but could not find their target (Fig. 3) These results indicate correlation between the *en* expression pattern and the final polarity of the tectum.

Ectopic tectum differentiated by heterotopic transplantation of the tectum anlage

We have mentioned that the tectum rostrocaudal polarity is not determined at E2, and it is regulated by the surrounding tissue. On the other hand, the fate of the tectum anlage is already determined by the 10-somite stage, and it differentiates as an optic tectum even when transplanted ectopically into the diencephalon or into the metencephalon (Nakamura, 1990). Ectopic tectum which differentiated at the diencephalon can receive retinal fibers (Alvarado-Mallart and Sotelo, 1984).

We studied the rostrocaudal polarity formation in the ectopic tectum produced in the diencephalon (Fig. 2B). First, E2 tectal anlage was transplanted into the E2 diencephalon. In the host tectum, *en* was expressed strongly at the caudal part and weakly at the rostral part. On the other hand, in the ectopic tectum, *en* expression was weak at the caudal part and strong at the rostral part. The further from the diencephalo-mesencephalon junction, the stronger the *en* expression (Fig. 4). Consequently, the pattern of *en* expression in the host and in the ectopic tecta was nearly a mirror image, suggesting the existence of a repressive influence on *en* expression around the diencephalo-mesencephalon junction (Itasaki *et al.*, 1991).

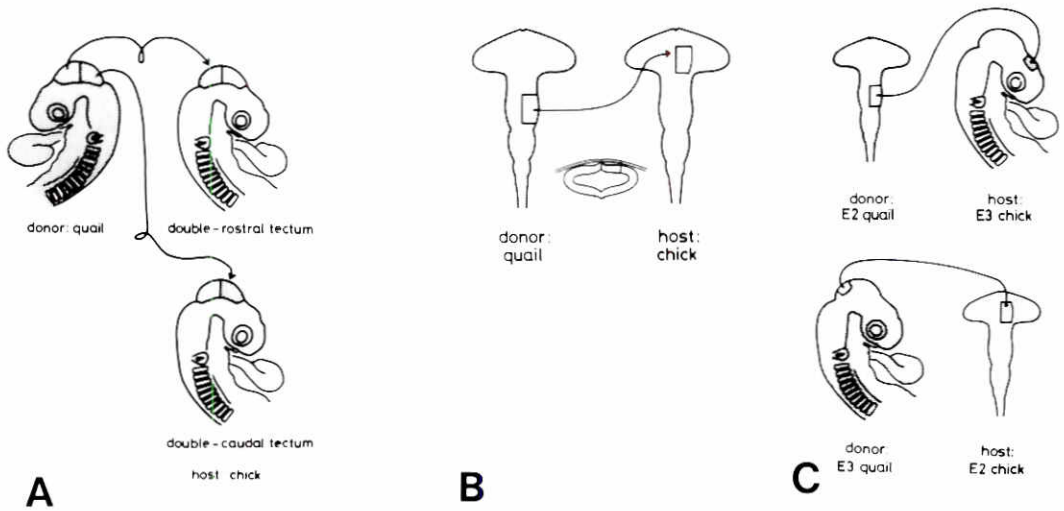

Fig. 2. Schematic drawings of the transplantation. *To identify the transplant, transplantation was carried out between quail and chick embryos.* **(A)** *Rostral half of the quail left mesencephalon was transplanted into the caudal half of the chick embryo at E3 to make double rostral tectum (upper). In a similar way, double caudal tectum was produced (lower).* **(B)** *Alar plate of E2 quail mesencephalon was transplanted into E2 chick diencephalon to produce ectopic tectum in the diencephalon.* **(C)** *Ectopic tectum was produced in the diencephalon between E2 and E3 embryos.*

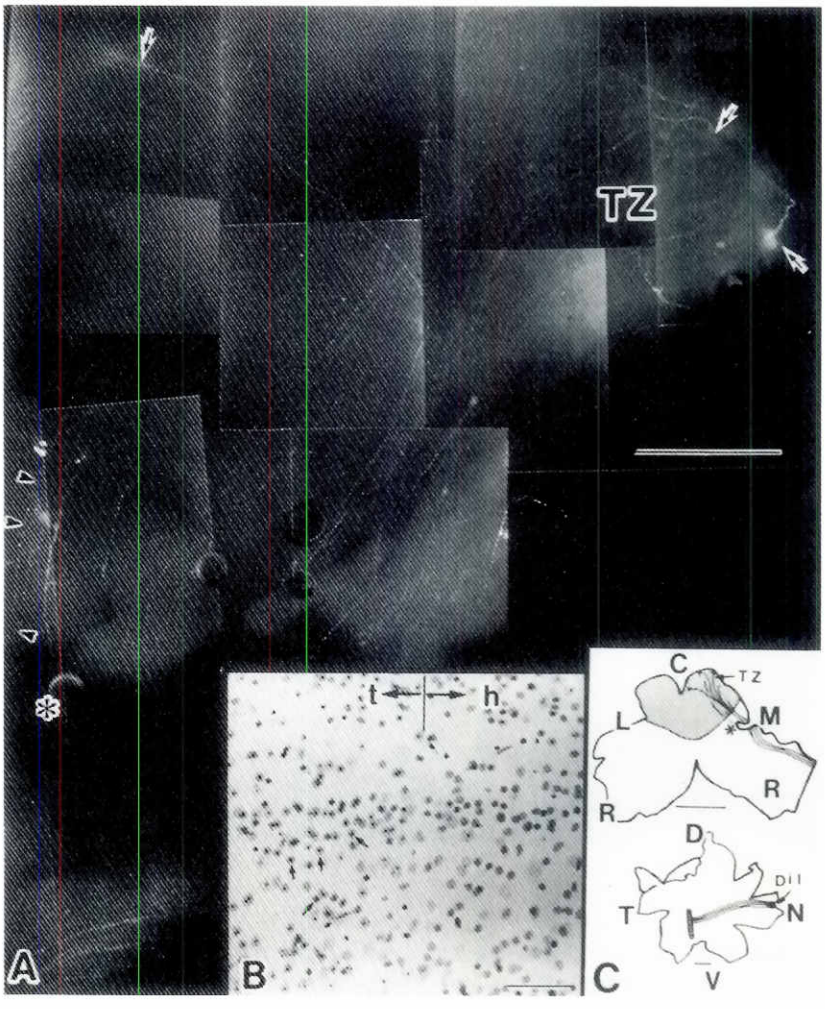

Fig. 3. Trajectories of nasal retinal fibers on the double rostral tectum. *Transplantation was performed between 24- and 26-somite stage quail and chick embryos. Nasal retinal fibers extended to the caudal pole of the double rostral tectum* **(A, C)**, *but they could not make terminal arborizations. Instead they are wandering (arrows in* **A**) *as if to find the target near the expected target zone (TZ).* **(B)** *shows the boundary between the host (h) and the transplant (t). The transplant is easily identified because quail cells have condensed heterochromatin (arrows).* **(C)** *represents camera lucida drawing of the tectum (upper) and retina (lower). Shadowed area represents the transplant. C: caudal, R: rostral, M:medial, L: lateral, D:dorsal, T: temporal, N: nasal, V: ventral, DiI, place where DiI was put.*

E7 ectopic tecta had the laminar structure characteristics of the developing tectum. Cytoarchitectonic development of the ectopic tecta proceeded in a mirror image to the host tectum (Fig. 5). The caudal part of the ectopic tecta had thicker wall and more laminas than the rostral (Itasaki *et al.*, 1991). This result suggests correlation between the *en* expression pattern and the pattern of cytoarchitectonic development. In both ectopic and host tecta, the place where the *en* is expressed most weakly differentiated faster than the place where *en* is most strongly expressed.

As mentioned earlier, the plasticity in the rostrocaudal polarity of the tectum is lost in the E3 embryo. It has also been suggested that the rostrocaudal polarity of the tectum is determined by the interaction of tectum and surrounding tissues. We pursued the mechanisms of polarity formation by heterochronic transplantation

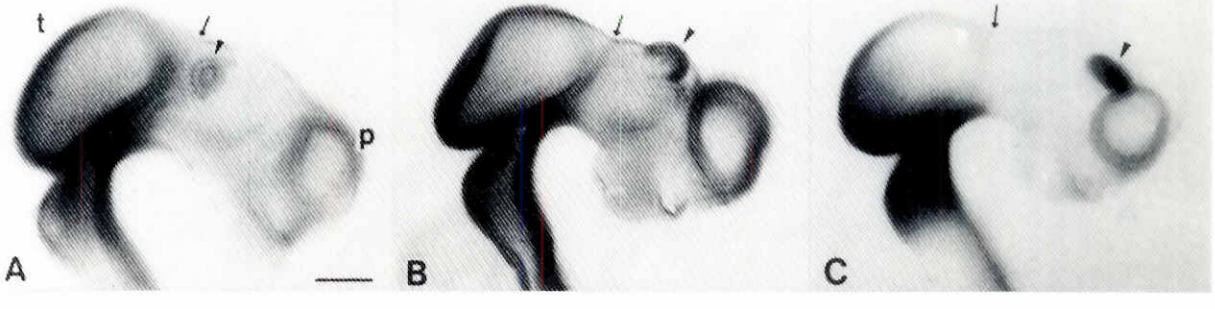

Fig. 4. Chimeras with ectopic tecta (arrow heads) at various positions in the diencephalon. *The further from the mes-diencephalon junction (arrows), the stronger the en expression was. This rule is also applicable to the host tectum. Bar, 500 μm.*

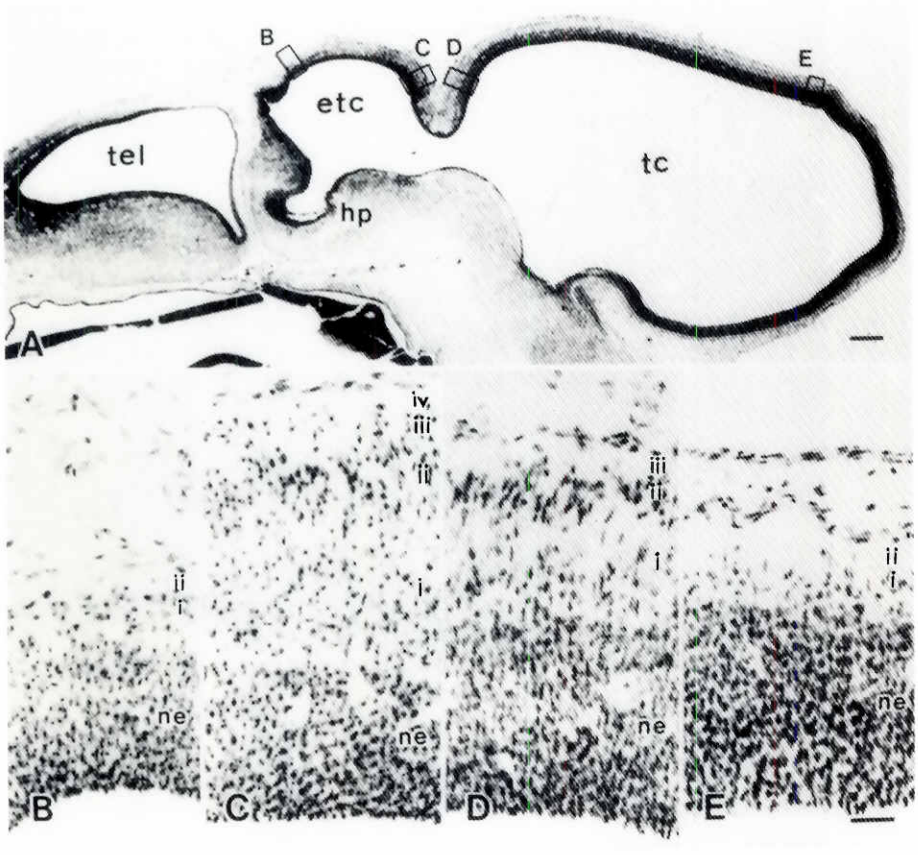

Fig. 5. Histology of chimeric brain with ectopic tectum. *In the host tectum, the rostral part* **(D)** *where en was more weakly expressed, differentiates faster than the caudal part* **(E)**. *On the other hand, in the ectopic tectum, caudal part* **(C)**, *where en expression was weaker, differentiates faster than the rostral* **(B)**. *Bar, A: 200 μm, B: 20 μm.*

(Itasaki *et al.*, 1991), i.e. we wanted to know whether E3 embryos lost plasticity in tectal rostrocaudal polarity formation because tectal anlage itself lost the competence or because the regulative activity was lost.

When the E3 tectal anlage was transplanted into the E2 diencephalon, the *en* expression pattern was reversed (Fig. 6A), i.e. *en* expression was strong at the rostral and weak at the caudal (near the diencephalo-mesencephalon junction), just as in the chimeric embryos produced by the transplantation between E2 donor and E2 host embryos. In other words, E3 tectal anlage changed the *en* expression pattern by regulative influence from the host embryo. On the other hand, when the E2 tectal anlage was

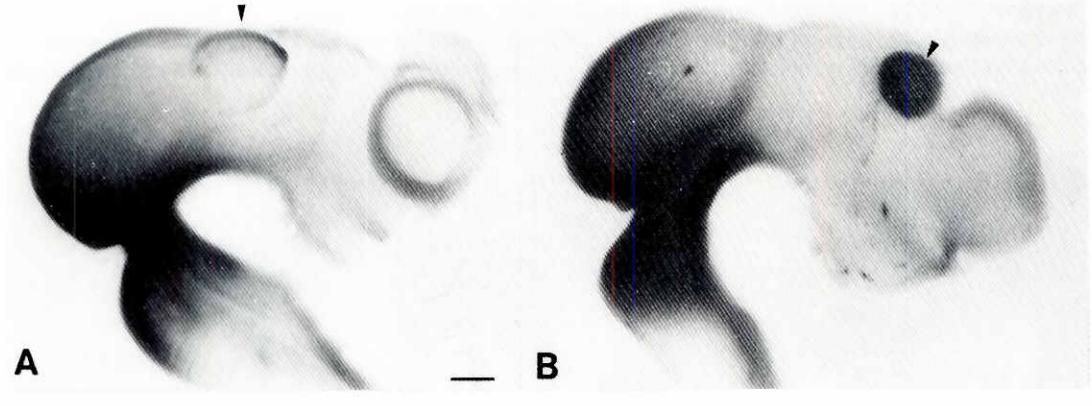

Fig. 6. Chimeras after heterochronic transplantations, stained with anti-*en* antibody. (A) *A chimera after transplantation of E3 mesencephalon into the E2 diencephalon.* **(B)** *A chimera after transplantation of E2 mesencephalon into the E3 diencephalon. Ectopic tectum in A has had its en expression pattern reversed, while the ectopic tecta in B kept its original pattern of en expression. This finding indicates that the E3 tectum anlage is competent to respond to regulating factors for rostrocaudal polarity, whereas the E3 embryo may have lost the regulating factors. Bar, 500 μm.*

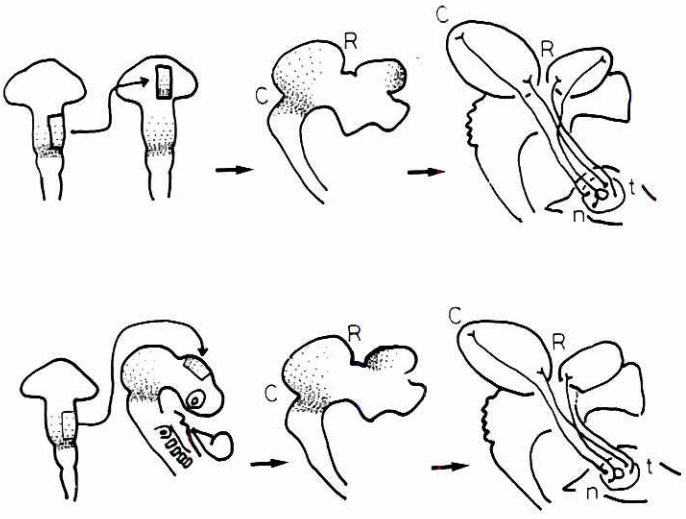

Fig. 7. Summary in the ectopic tectum. *When E2 mesencephalon is transplanted into the E2 diencephalon (upper), en expression is a mirror image to the host tectum. Subsequent developmental events proceed in a mirror image to the host. The caudal part of the ectopic tectum may have acquired the 'rostral' property and receives nasal retinal fibers, and rostral part of the ectopic tectum receives temporal retinal fibers. When E2 mesencephalon is transplanted into the E3 diencephalon (lower), the transplant keeps its original fate of en expression pattern. Caudal part of the ectopic tecta, in this case, receives nasal retinal fibers. C: caudal, R: rostral, t: temporal, n: nasal.*

transplanted into the E3 diencephalon, the transplant kept its original pattern of *en* expression, i.e. strong at the caudal and weak at the rostral part (Fig. 6B). These results indicate that E3 tectal anlage has the competence to respond to the regulative influence, but E3 embryo lost the regulative activity of tectal rostrocaudal polarity formation.

Retinal fiber projection to the ectopic tecta

By labeling whole retinal fibers by HRP (Alvarado-Mallart and Sotelo, 1984), it was shown that retinal fibers invade the ectopic tecta produced in the diencephalon. Topographic order of retinal fiber projection on the ectopic tecta was examined by labeling a tiny part of the retina by fluorescent dye, DiI or by DiA (Itasaki and Nakamura, 1992).

In a normal retinotectal projection map, nasal retinal ganglion cells project to the caudal tectum, and temporal retinal ganglion cells project to the rostral tectum. On the ectopic tecta, nasal retinal fibers made terminal arborizations on the rostral part, and temporal retinal fibers terminated at the caudal part (Itasaki and Nakamura, 1992). In other words, nasal retinal fibers terminated at the place where *en* expression had been strong, and temporal retinal fibers terminated at the place where *en* had been expressed weakly (Fig. 7). To summarize the results, the *en* expression pattern of ectopic tecta produced between E2 host and donor was a mirror image to that of the host tecta, and subsequent development proceeded in a mirror image to the host.

Ectopic tecta produced by transplantation of E2 mesencephalon into E3 diencephalon kept the original *en* expression pattern, i.e. in ectopic tecta, *en* expression was stronger at the caudal part than at the rostral part. On this kind of ectopic tecta, nasal retinal fibers made terminal arborizations at the caudal part of the ectopic tecta (Fig. 7) (Itasaki and Nakamura, 1992).

Our experimental results on rostrocaudal polarity all show the consistent relation between the *en* expression pattern and subsequent development including the retinotectal projection pattern. When the *en* expression pattern is reversed in the ectopic environment, subsequent cytoarchitectonic development and retinotectal projection patterns were in reverse to the host.

Concluding remarks

Our study series showed close correlation between *en* expression pattern and subsequent tectal polarity including the polarity to receive retinal fibers. It may be interesting to look at retinal projection patterns after inducing *en* expression ectopically by expression vectors such as retrovirus. Such experiments are being carried out in our lab.

en is a homeobox containing gene originally identified as a segment polarity gene in *Drosophila* (Kornberg, 1981). In *Drosophila*, many genes which are involved in morphogenesis have been identified. Recent studies have shown that the homolog of such genes are active in vertebrate development. *en* is one such gene. *wnt-1*, which is a homolog of *wingless (wg)* in *Drosophila*, has been shown to be essential for mesencephalon and cerebellum development in mice by the gene targeting method (McMahon and Bradley, 1990; Thomas and Capecchi, 1990). In *Drosophila*, it has been shown that *en* and *wnt-1* mutually interact for their expression (Martinez-Arias, *et al.*, 1988). Thus it may be interesting to elucidate genes which regulate *en* expression in chicken mesencephalon. It may be also interesting to study what gene *en* regulates because *en* is a homeobox containing gene.

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